

Docket No.: 1254-0281PUS1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Noriaki HATTORI et al.

Application No.: 10/829,250

Confirmation No.: 8196

Filed: April 22, 2004

Art Unit: 1652

For: LUCIFERASE AND METHODS FOR
MEASURING INTRACELLULAR ATP USING
THE SAME

Examiner: E. Slobodyansky

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Mr. Seiji Murakami, hereby declare as follows in connection with the above-referenced U.S. Patent application.

1. I am a citizen of Japan, presently receiving mail at c/o Kikkoman Corporation, Takasago Factory, 1-1, Shinhami 1-chome, Arai-cho, Takasago-Shi,

Hyogo 676-8510 Japan.

I have trained in biochemistry at (insert description of academic training and work experience, and present position of employment – here we are establishing Mr. Murakami's credentials as a biochemist. A copy of his Curriculum Vitae may be attached.)

2. I am a co-inventor of the subject matter claimed in the instant application. As such, I am familiar with the disclosure of the application, its claims and its prosecution history.

3. The Examiner has cited U.S. Patent 6,074,859 as prior art against the instant claims

14-18, 20-24, 32 and 35-41 under 35 USC § 102(e) of the U.S. patent statutes. I am a co-inventor of the subject matter of the '859 patent. As such, I am familiar with its disclosure, prosecution history and claims.

4. As indicated by its title, the '859 patent discloses and claims mutant bioluminescent proteins. Among the proteins disclosed are firefly luciferases having a mutation (i.e. an amino acid other than glutamic acid) at a position corresponding to position 490 of GENJI or HEIKE firefly luciferase (SEQ ID NO: 14).

5. The '859 patent examines the activity of the mutant bioluminescent proteins in a variety of buffers; among them several organic acids and zwitterionic compounds, for example MES, HEPES, TAPS, CHES and CAPS. The activity of a mutant luciferase in solutions of these buffers is assayed as a function of pH. See, for example, Example 5 and the data in Figure 1 of the '859 patent.

6. The '859 patent is silent as to the activity of the mutant enzymes disclosed in surfactant solutions.

7. The claims of the '859 patent do not specifically recite mutation of a protein at a position corresponding to position 490 of a GENJI or HEIKE firefly luciferase. On the other hand, claim 1 of the '859 patent specifically recites mutation of a firefly luciferase at position 219 and claim 2 of the '859 patent specifically recites mutation of a firefly luciferase at position 290.

8. In view of paragraphs 6. and 7. above, the '859 patent must be viewed as disclosing, but not claiming, a bioluminescent protein that includes a mutation at a position corresponding to position 490 of a GENJI or HEIKE firefly luciferase.

9. The making of the mutant luciferase SEQ ID NO: 14 is described in Example 5 of the '859 patent. I am the person who designed this experiment and directed that it be performed (performed?) it. I am the person who determined the sequence of the resulting mutant luciferase, and therefore I am the person who conceived and reduced to practice this mutant enzyme.

10. I am the person who directed that the mutant enzyme SEQ ID NO: 14 be assayed for activity as a function of pH in the solutions of the various buffers as described in Example 5, the results being shown in Table 6 and Figure 1 of the '859 patent. Thus, I conceived and reduced to practice that the enzyme of SEQ ID NO: 14 is one that retains most of its bioluminescent activity in various amine-containing buffers at a range of pH.

11. The Examiner of the instant application explains at page 12 of the Office Action mailed June 15, 2007 that "Both Hirokawa et al. sequences have E490K substitution. Said mutant luciferase has an improved activity compared with the wild-type luciferase in buffers containing surfactants. Hirokawa et al. teach methods for measuring ATP using luciferase (Example 5)" These statements include two errors of fact. First, only SEQ ID NO: 14 among those disclosed in the '859 patent includes the E490K mutation. Second, the solutions in which the activity of the bioluminescent proteins was assayed do not contain surfactants.

12. The luciferase assay utilized in the '859 patent is disclosed at column 6, lines 59-64. At that part of the disclosure, HEPES is used as a buffer. No compound disclosed is a surfactant. The various other buffers used in the tests of Example 5 of the '859 patent are disclosed at column 12, lines 55-62.

13. One of ordinary skill in the art of biochemistry would not consider any of the buffers disclosed in the '859 patent, and in particular the MES, HEPES, TAPS, CHES or CAPS buffers shown in Figure 1, to be surfactants. Exhibits A-D attached provide evidence that supports this

conclusion.

Exhibit A is a copy of a chemical dictionary published in Japan (Chemical Dictionary, edited by Michinori Ohgi et al., published by TOKYO KAGAKU DOJIN, on October 1, 1994, 9 p. 249). It describes "The features of surfactants are that the molecule of the surfactant is composed of hydrophilic group and lipophilic group (hydrophobic group), the hydrocarbon group which is lipophilic group has some length e.g. more than eight carbons),....." at lines 27 to 30 in the left column on page 249.

Exhibit B is a copy of a page on the WIKIPEDIA website (<http://en.wikipedia.org/wiki/Surfactant>). It describes "The term surfactant is a blend of 'surface active agent' ". Surfactants are usually organic compounds that are amphipathic, meaning they contain both hydrophobic groups (their "tails") and hydrophilic groups (their "heads"). Therefore, they are soluble in both organic solvents and water."

MES, HEPES, TAPS, CHES and caps are ingredients of Good's buffer (Good, N.E. et al: Biochemistry 5, 467 (1966); Good, N.E. & Izawana, S.: Methods Enzymol., Part B, Vol. 24, p. 53 ff. (Pietro, ed.) (1972) Academic Press, New York). Exhibit C is a copy of a page explaining Good's buffer in a website of DOJINDO LABORATORIES which is Japanese reagent vendor. It describes five features of Good's buffers. One of five is that they have very low permeability through biological membranes. This means that Good's buffers are difficult to dissolve in organic solvents.

Exhibit D is copies of pages explaining the features and structure of MES, HEPES, TAPS, CHES and CAPS. As Exhibit D demonstrates, MES, HEPES, TAPS, CHES and CAPS are not composed of a hydrophilic group and lipophilic group (hydrophobic group). Exhibit D also describes that MES, HEPES, TAPS and CAPS are not soluble in organic solvents.

14. I hereby declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under

Application No. 10/828,280
Declaration under 37 CFR 1.132 of Seiji Muralami
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Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted this 12 day of December, 2007

By Seiji Muralami
Seiji Muralami

化学辞典

編 集

大木道則 大沢利昭
田中元治 千原秀昭

Chemical Dictionary

東京化学同人

電位の差 $e\phi_0 - e\phi = \mu^0 - \mu$ はボルツマン電位差 (Volta potential difference) とよばれ、測定可能な量である。接続していい二つの相 α, β の阿で化学種 B が平衡にあるとき、二相のボルツマン電位差は下より接線間電位差^{*}とよばれる。

外部標準 [external standard] 物質分析、ことに元素分析や原子分光又は吸収分析などにおいて、試料の前処理

また、液相中に分散・浮遊しているような場合であつても、後者の場合は分散するコロイド粒子が多く、その総面積が非常に大きくなることから、接する界面の比容全体に性質を決定するほどに大きく影響する。罪よ、よび表面に於いては、内相での物理的、化学的性質は分解できなない特異的な現象が起こり、内部の性質

界面でもその界面の面積を縮小する方向に働く張力をいふ。
一般に界面張力は、不溶性であるかきそはわずかに溶け合
ひあつた二つの液体の界面で現れる。気/液、気/固界面ではよつ
と表面張力がよばれる。

海绵鉄 [sponge iron] = 還元鉄

界面電位差 [interfacial potential (difference)] = 二

X

Xe(キセノン) 323 a
XeF₂ 1224 a
XeF₄ 1224 a
XeF₆ 1224 a
XeF₂O₃ 1224 b
XeF₄O 1224 b
XeO₃ 524 b
XeO₄ 524 b
Xe⁺[PtF₆]⁻ 1227 b

Y

Y(イットリウム) 124 b

YCl₃ 196 b, 328 b
Y(NO₃)₃ 674 b
Y₂O₃ 329 a, 522 b
Yb(イットリウム) 124 a
YbCl₃ 196 b, 328 b
Yb(NO₃)₃ 674 b
Yb₂O₃ 329 a
Z
Zn(亜鉛) 4 a
ZnBr₂ 647 b
Zn(CH₃)₂ 653 b
Zn(C₂H₅)₂ 660 b
Zn[CH₂CH(OH)COO]₂ 1034 b
Zn(CH₃COO)₂ 509 b
ZnCl₂ 197 b
Zn(FeO₂)₂ 521 b
ZnI₂ 1468 a
ZnO 621 b
ZnO₂ 267 b
ZnS 1514 a
ZnSO₄ 1519 a
Zr(ジルコニウム) 689 b
ZrC 814 b
[Zr(C₆(CH₃)₆)₂] 1135 b
[Zr(C₆H₆)₂] 1132 b
ZrCl₄ 202 a
[ZrCl₂(C₆H₅)₂] 587 a
ZrCl₂O 1010 b
ZrI₄ 1471 a
ZrO₂ 626 b
Zr(OH)₄ 703 b
ZrO(NO₃)₂ 1019 b
Zr(SO₄)₂ 1522 a
ZrSiO₄ 236 b

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化学辞典

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田中元治・千原秀昭

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Surfactant

Exhibit B

From Wikipedia, the free encyclopedia

This article is about surfactants in general. For the compound produced by alveolar cells, see pulmonary surfactant.

Surfactants, also known as **tensides**, are wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids.

Contents

- 1 Etymology
- 2 Operation and effects
- 3 Applications and sources
- 4 Classification
- 5 See also

Etymology

The term *surfactant* is a blend of "surface acting agent". Surfactants are usually organic compounds that are amphipathic, meaning they contain both hydrophobic groups (their "tails") and hydrophilic groups (their "heads"). Therefore, they are soluble in both organic solvents and water. The term surfactant was coined by Antara Products in 1950.

In Index Medicus and the United States National Library of Medicine, "surfactant" is reserved for the meaning *pulmonary surfactant* (see "alveoli" link below). For the more general meaning, "surface active agent" is the heading.

The most common, biological example of surfactant is that coating the surfaces of the Alveoli, the small air sacs of the lungs that serve as the site of gas exchange.

Operation and effects

Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution into aggregates. Some of these aggregates are known as micelles. The concentration at which surfactants begin to form micelles is known as the critical micelle concentration or CMC. When micelles form in water, their tails form a core that can encapsulate an oil droplet, and their (ionic/polar) heads form an outer shell that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain favorable contact with oil. Surfactants are also often classified into four primary groups; anionic, cationic, non-ionic, and zwitterionic (dual charge).

Thermodynamics of the surfactant systems are of great importance,

A micelle - the lipophilic ends of the surfactant molecules dissolve in the oil, while the hydrophilic charged ends remain outside, shielding the rest of the hydrophobic

theoretically and practically. This is because surfactant systems represent systems between ordered and disordered states of matter. Surfactant solutions may contain an ordered phase (micelles) and a disordered phase (free surfactant molecules and/or ions in the solution).

micelle

Ordinary washing up (dishwashing) detergent, for example, will promote water penetration in soil, but the effect would only last a few days (although many standard laundry detergent powders contain levels of chemicals such as sodium and boron, which can be damaging to plants, so these should not be applied to soils). Commercial soil wetting agents will continue to work for a considerable period, but they will eventually be degraded by soil micro-organisms. Some can, however, interfere with the life-cycles of some aquatic organisms, so care should be taken to prevent run-off of these products into streams, and excess product should not be washed down gutters.

Applications and sources

Surfactants play an important role in many practical applications and products, including:

- Detergents
- Fabric softener
- Emulsifiers
- Paints
- Adhesives
- Inks
- Anti-fogging
- Soil remediation
- Wetting
- Ski Wax
- Snowboard Wax
- Foaming
- Defoaming
- Laxatives
- Agrochemical formulations
 - Herbicides
 - Insecticides
- Quantum dot coating
- Biocides (Sanitizers)
- Hair Conditioners (after shampoo)
- Spermicide (Nonoxynol 9)
- Used as an additive in 2.5 gallon fire extinguishers

Surfactants are also naturally secreted by type II cells of the lung alveoli in mammals.

Classification

A surfactant can be classified by the presence of formally charged groups in its head. A nonionic surfactant has no charge groups in its head. The head of an ionic surfactant carries a net charge. If the charge is negative, the surfactant is more specifically called anionic; if the charge is positive, it is called cationic. If a surfactant contains a head with two oppositely charged groups, it is termed zwitterionic.

Some commonly encountered surfactants of each type include:

- Ionic

- Anionic (based on sulfate, sulfonate or carboxylate anions)
 - Sodium dodecyl sulfate (SDS), ammonium lauryl sulfate, and other alkyl sulfate salts
 - Sodium laureth sulfate, also known as sodium lauryl ether sulfate (SLES)
 - Alkyl benzene sulfonate
 - Soaps, or fatty acid salts
- Cationic (based on quaternary ammonium cations)
 - Cetyl trimethylammonium bromide (CTAB) a.k.a. hexadecyl trimethyl ammonium bromide, and other alkyltrimethylammonium salts
 - Cetylpyridinium chloride (CPC)
 - Polyethoxylated tallow amine (POEA)
 - Benzalkonium chloride (BAC)
 - Benzethonium chloride (BZT)
- Zwitterionic (amphoteric)
 - Dodecyl betaine
 - Dodecyl dimethylamine oxide
 - Cocamidopropyl betaine
 - Coco ampho glycinate
- Nonionic
 - Alkyl poly(ethylene oxide)
 - Copolymers of poly(ethylene oxide) and poly(propylene oxide) (commercially called Poloxamers or Poloxamines)
 - Alkyl polyglucosides, including:
 - Octyl glucoside
 - Decyl maltoside
 - Fatty alcohols
 - Cetyl alcohol
 - Oleyl alcohol
 - Cocamide MEA, cocamide DEA, cocamide TEA

See also

- Anti-fog

Retrieved from "<http://en.wikipedia.org/wiki/Surfactant>"

Categories: Colloidal chemistry | Cleaning product components | Surfactants

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用途：13. 生化学用緩衝剤
検索用頭文字：A

Exhibit L

同仁品コード：GB01 製品名：ACES

題名：Good's Buffersの特長は？

Q：Good's Buffersの特長は何ですか？

A：-Good's Buffer特長-

Features of Good's Buffer

- 1) 水に良く溶け、濃厚な緩衝液が作成できる
- 2) 生体膜を透過しにくい
- 3) 酸解離平衡が濃度、温度、イオン組成の影響を受けにくい
- 4) 金属イオンとの錯形成能が小さい
- 5) 化学的に安定で、再結晶による高純度精製が可能
- 6) 可視、紫外部に吸収を持たないために、目的成分の検出が容易

2) They have low permeability through biological membrane.

最適pH範囲がそれぞれ異なりますので、目的のpHのものをご使用ください。

pKa(20°C)	グッド緩衝	利用最適pH範
6.15	MES	5.5-7.0
6.46	Bis-Tris	5.7-7.3
6.80	ADA	5.8-7.4
6.80	PIPES	6.1-7.5
6.90	ACES	6.0-7.5
6.95	MOPSO	6.2-7.4
7.15	BES	6.6-8.0
7.20	MOPS	6.5-7.9
7.50	TES	6.8-8.2
7.55	HEPES	6.8-8.2
7.60	DIPSO	6.9-8.1
7.70	TAPSO	7.0-8.2
7.85	POPSO	7.2-8.5
7.90	HEPPSO	7.4-8.6
8.00	EPFS	7.5-8.5
8.15	Tricine	7.8-8.8
8.35	Bicine	7.7-9.1
8.40	TAPS	7.7-9.1
9.50	CHES	8.6-10.0
10.00	CAPSO	9.3-10.7
10.40	CAPS	9.7-11.1

*回答はいかがでしたか？

顧客満足を高めるためにアンケートを実施しております。

よろしければご協力ください。

URL:<http://www.dojindo.co.jp/cs/cs.html>

○Q&Aの内容で不十分な場合、下記にお問い合わせ下さい。

(株)同仁化学研究所 カスタマーサービス部

Free dial: 0120-489548 Free fax: 0120-021557

E-mail: info@dojindo.co.jp

同仁品コード:GB12

CAS No.[4432-31-9(anhydrous),
145224-94-8(monohydrate)]

13.生化学用緩衝剤 - 最適pH範囲:5.5~7.0

MES

化学名 2-Morpholinooethanesulfonic acid, monohydrate

25g	¥2,730 (本体価格: ¥2,800)	341-01622
100g	¥6,720 (本体価格: ¥6,400)	349-01623
250g	¥14,700 (本体価格: ¥14,000)	343-01621
500g	¥27,300 (本体価格: ¥26,000)	345-01625
1kg	¥49,350 (本体価格: ¥47,000)	343-01626

性質 水には溶けるが、TES、HEPES に比較すれば溶解度は小さく、0.65 mol/l(0℃)で飽和する。有機溶媒には溶けない。 $pK_a=6.15$ 、pH5.5~7.0の緩衝液を作るのに適する。

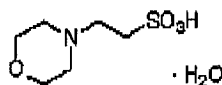
MESはグッド緩衝剤(Good's buffer:グットバッファー)の一つで、代表的な緩衝剤である。細胞培養、組織培養など生化学分野で広く使用されている。

規格

(1) 性状:本品は白色結晶性粉末で、水に溶ける。(2) 純度(滴定):99.0% 以上 (3) 水溶状:
試験適合 0.020 以下(300 nm) (4) 乾燥減量(110℃):6.0~9.0% (5) 強熱残分(硫酸塩):0.10%
以下 (6) 重金属(Pbとして):0.0005% 以下 (7) 鉄(Fe):0.0005% 以下
溶解例 2.1 g/10 ml(水)

MES

It is not
solved in organic
solvents.




$C_6H_9NO_4S \cdot H_2O=213.25$

参考文献

MSDS

MES MES、細胞培養、組織培養、生化学緩衝剤、グッド緩衝剤、グットバッファー

 (ホームページへはロゴをクリック)

同仁品コード:GB10

CAS No.[7365-45-9]

06.細胞増殖/細胞毒性測定用試薬—関連試薬,13.生化学用緩衝剤—最適pH範囲:6.8~8.2

HEPES

化学名 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid

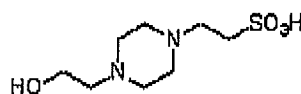
25g	¥2,520 (本体価格: ¥2,400)	348-01372
100g	¥6,720 (本体価格: ¥6,400)	346-01373
250g	¥14,700 (本体価格: ¥14,000)	340-01371
500g	¥24,150 (本体価格: ¥23,000)	342-01375
1kg	¥46,410 (本体価格: ¥44,200)	340-01376

性質 水によく溶け、2.25 mol/l(0℃)で飽和する。有機溶媒にはほとんど溶けない。 $pK_a=7.55$ 、
pH6.8~8.2の緩衝液を作るのに適する。

HEPESはグッド緩衝剤(Good's buffer:グットバッファー)の一つで、その中でも代表的な緩衝剤である。細胞培養、組織培養など生化学分野で広く使用されている。

規格

(1) 性状:本品は白色結晶性粉末で、水に溶ける。(2) 純度(滴定):99.0% 以上 (3) 水溶状:
試験適合 0.025 以下(320 nm) (4) 乾燥減量(110℃):0.20% 以下 (5) 強熱残分(硫酸塩):0.10%
以下 (6) 重金属(Pbとして):0.0005% 以下 (7) 鉄(Fe):0.0005% 以下
溶解例 2.4 g/10 ml(水)

CaH₁₈N₂O₄S=238.31

参考文献

MSDS

HEPES HEPES、細胞培養、組織培養、生化学緩衝剤、グッド緩衝剤、グットバッファー

(ホームページへはロゴをクリック)

HEPES

It is not almost
solved in organic
solvents.

同仁品コード:GB17

CAS No.[29915-38-8]

13.生化学用緩衝剤 - 最適pH範囲:7.7~9.1

TAPS

TAPS

化学名 *N*-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid

25g

¥3,360 (本体価格: ¥3,200)

344-02572

100g

¥9,870 (本体価格: ¥9,400)

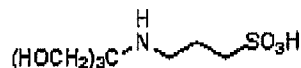
340-02574

性質 水にはかなりよく溶けるが、有機溶媒には溶けない。 $pK_a=8.40$ 、 $pH7.7\sim9.1$ の緩衝液を作るのに適する。

It is not
solved in
organic solvents.

規格

(1) 性状:本品は白色結晶性粉末で、水に溶ける。(2) 純度(滴定):99.0% 以上 (3) 水溶状:
試験適合 0.025 以下(300 nm) (4) 乾燥減量(110°C):0.40% 以下 (5) 強熱残分(硫酸塩):0.10%
以下 (6) 重金属(Pbとして):0.0005% 以下 (7) 鉄(Fe):0.0005% 以下
溶解例 2.43 g/10 ml(水)

 $C_7H_{17}NO_6S=243.28$ 

参考文献

MSDS

TAPS



(ホームページへはロゴをクリック)

同仁品コード:GB07

CAS No.[103-47-9]

13.生化学用緩衝剤 - 最適pH範囲:8.6~10.0

CHES

CHES

化学名 *N*-Cyclohexyl-2-aminoethanesulfonic acid

25g

¥3,990 (本体価格: ¥3,800)

342-04692

性質 水に溶ける。p*K*_a=9.5、pH8.6~10.0の緩衝液を作るのに適する。

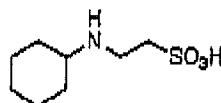
規格

(1) 性状:本品は白色結晶性粉末で、水に溶ける。(2) 純度(滴定):99.0% 以上 (3) 水溶状:

試験適合 0.025 以下(300 nm) (4) 乾燥減量(110℃):0.20% 以下 (5) 強熱残分(硫酸塩):0.10%

以下 (6) 重金属(Pbとして):0.0005% 以下 (7) 鉄(Fe):0.0005% 以下

溶解例 2.1 g/10 ml(水)



C₆H₁₁NO₃S=207.29

参考文献

MSDS

CHES



(ホームページへはロゴをクリック)

同仁品コード:GB06

CAS No.[1135-40-6]

13.生化学用緩衝剤 - 最適pH範囲:9.7~11.1

CAPS

化学名 *N*-Cyclohexyl-3-aminopropanesulfonic acid

25g

¥4,200 (本体価格: ¥4,000)

347-00482

100g

¥11,340 (本体価格: ¥10,800)

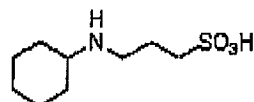
343-00484

性質 水に溶け、0.8 mol/l(0°C)で飽和する。有機溶媒には溶けない。 $pK_a=10.40$ 、pH9.7~11.1の緩衝液を作るのに適する。

規格

(1) 性状:本品は白色結晶性粉末で、水に溶ける。(2) 純度(滴定):99.0% 以上 (3) 水溶状:試験適合 0.030 以下(270 nm) (4) 乾燥減量(110°C):0.50% 以下 (5) 強熱残分(硫酸塩):0.10% 以下 (6) 重金属(Pbとして):0.0005% 以下 (7) 鉄(Fe):0.0005% 以下
溶解例 2.2 g/10 ml(水)

It is not
solved in
organic solvents.



$C_6H_{11}NO_3S=221.32$

参考文献

MSDS

CAPS



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